GENOTYPING OF GLOBAL *YERSINIA PESTIS* ISOLATES BY USING IS*285*

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ABSTRACT

Yersinia pestis is the etiologic agent of bubonic and pneumonic plague, one of the most dangerous bacterial infections. Plague is a re-emerging disease displaying current tendency to increasing reports of human cases, including the affliction with multidrug-resistant strains of *Y. pestis*. The plague bacterium is a potential agent of biowarfare and bioterrorism. Therefore, both military and civilian specialists should have efficient methods of molecular identification of *Y. pestis* strains and their assignment to certain ecological variants. In this work, we consider literature data, as well as our previous and new results on genotyping of global *Y. pestis* strains. We come to conclusion that a mobile genetic element, IS285, is one of the most powerful molecular tools allowing to trace the circulation of epidemic clones and to detect their geographical/animal origin.

1. INTRODUCTION

Yersinia pestis is the causative agent of plague circulating in natural foci among about 200 species of rodents and lagomorphs. Humans usually become infected from animals via fleabites and display the bubonic form of plague, but in the case of secondary affect on lungs, a pneumonic disease occurs that is particularly highly lethal and can be easily transmitted from person to person through infected aerosols (Perry, Fetherston, 1997; Titball *et al.*, 2003; Anisimov *et al.*, 2004; Lindler, 2005).

Plague seems to have killed about 200 million people during three pandemics and now is recognized as a re-emerging disease, due to increasing reports of human plague cases, return of the disease to areas where no cases were observed for a few decades, and appearance of multidrug-resistant strains of *Y. pestis*. These factors make *Y. pestis* a potential agent of biowarfare and bioterrorism belonging to the most dangerous category of pathogens, CDC group A (Madan, 1995; Barreto *et al.*, 1995; Galimand *et al.*, 1997; Inglesby *et al.*, 2000; Boisier *et al.*, 2002; Gage, Kosoy, 2005; Migliani *et al.*, 2006).

Y. pestis is considered a recently emerged clone of *Yersinia pseudotuberculosis* (Achtman *et al.*, 1999). Using the characters of glycerol fermentation and nitrate reduction, *Y. pestis* was divided into biovars Antiqua, Medievalis, and Orientalis (Devignat, 1951). Antiqua strains are isolated from marmots in the territories of Central Africa, Central and North Asia. Another continental biovar, Medievalis, is spread among ground squirrels and gerbils in Southeastern Europe, Central Asia and some desert regions of Africa. "Oceanic" isolates of biovar Orientalis circulate mainly on rats in South and Southeast Asia, Southern Africa and both Americas. Prevalence of Orientalis strains in Western culture collections and lack of nucleotide polymorphism in some of *Y. pestis* housekeeping genes gave rise to the opinion on limited phenotypic and genetic diversity of this species (Achtman *et al.*, 1999; 2004; Torrea *et al.*, 2006). However, *Y. pestis* diversity seems to be underestimated. E.g., there are several new taxons within this species yet to be investigated on the molecular level, so called pestoides strains. They are isolated in Southeastern Europe, Central and North Asia and some regions of Africa. Pestoides strains display unusual abilities to ferment rhamnose and melibiose and avirulence for guinea pigs being virulent for mice and wild rodents, their natural hosts (Martinevskii, 1969; Aparin, Golubinskii, 1989; Anisimov *et al.*, 2004).

To develop a comprehensive intraspecific classification of *Y. pestis*, as well as to trace circulation of epizootic and epidemic clones, including isolates used as a biological weapon, one should have efficient tools of genotyping. In this work, we analyze literature data and present our results of *Y. pestis* genotyping. Our previous publications and new experiments with global isolates suggest that IS*285* typing is the optimal method for establishing philogenetic relationships between *Y. pestis* strains, their potential geographical and animal origin.

2. GENOTYPING OF *Y. PESTIS* – CLUSTERING AND DISCRIMINATION

There are several methods of molecular typing of *Y. pestis* differing in their clustering and discrimination

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activities. The former means detection of phylogenetic relations among strains and assignment of them to discrete intraspecific taxons. The latter ability is referred to differentiation of closely related strains.

Some of the methods showed a good clustering power. For example, plasmid content typing of 257 *Y. pestis* strains isolated around the world was based on the presence and sizes of three virulence plasmids (pPst, pLcr, and pFra), as well as of various cryptic replicons. We established 20 new taxons, plasmidovars, widely ranging in their prevalence. One of them contained almost the whole biovar Orientalis, others corresponded to certain natural plague foci and some plasmidovars were represented by single unique strains (Filippov *et al.* 1990b; Filippov, 2001).

The methods of ribotyping (Guiyoule *et al.,* 1994; 1997; Panda *et al.,* 1996) and analysis of plasmid pLcr restriction fragment length polymorphism, RFLP (Bobrov, Filippov, 1998), allowed grouping of *Y. pestis* strains according to their geographical origin and was in agreement with the division of the species into biovars Antiqua, Medievalis, and Orientalis. However, these methods had insufficient discrimination abilities. For instance, ribotyping of 70 *Y. pestis* strains resulted in elucidation of 16 ribotypes. Two of them (B and O) included 66% of the isolates tested, whereas the 14 other ribotypes were found in no more than three strains each (Guiyoule *et al.,* 1994). Pulsed-field gel electrophoresis of chromosomal DNA cut with I-CeuI (Rakin, Heesemann, 1995) gave similar results. However, macrorestriction analysis with the use of other enzyme, SpeI, had a high discriminative power (Lucier, Brubaker, 1992; Huang *et al.,* 2002).

Techniques of profiling strains with short DNA repeats (Adair *et al.,* 2000; Klevytska *et al.,* 2001; Le Fleche *et al.,* 2001; Suchkov *et al.,* 2002; 2004; Achtman *et al.,* 2004; Girard *et al.,* 2004; Drancourt *et al.,* 2004; Pourcel *et al.,* 2004; 2005) usually provide good differentiation of isolates within the same biovar but in some cases seem to produce errors in clustering. Multiple locus VNTR (variable number tandem repeat) analysis (MLVA) detected 102 unique patterns in 104 strains of *Y. pestis* and *Y. pseudotuberculosis* but the phylogenetic dendrogram based on MLVA showed within the biovar Orientalis of *Y. pestis* five clusters isolated and remoted from each other. This finding contradicts the concept of global spread of Orientalis strains from Hong Kong during the third plague pandemic (Devignat, 1951), our data on belonging the vast majority of Orientalis strains to one plasmidovar (Filippov *et al.* 1990b; Filippov, 2001) and IS*100* typing results placing Orientalis isolates in close subgroups within the same cluster (Achtman *et al.,* 1999; Motin *et al.,* 2002; Torrea *et al.,* 2006).

The optimal combination of clustering and discriminative power of typing was found when mobile genetic elements IS*100* and IS*1541* were used (Simonet *et al.,* 1996; Bobrov, Filippov, 1997; Achtman *et al.,* 1999; 2004; Huang *et al.,* 2002; Motin *et al.,* 2002; Torrea *et al.,* 2006). For example, IS*100* RFLP analysis of 49 *Y. pestis* strains led to elucidation of 41 IS types, and all the strains formed three discrete clusters corresponding to the three biovars and certain subclusters correlating with the countries of isolation (Achtman *et al.,* 1999). However, IS typing has been carried out with small culture collections or they did not represent the global diversity of *Y. pestis* missing glycerol positive strains from Europe and the vast majority of Central and Eastern Asian plague foci.

3. IS*285* AND IS*285* TYPING

3.1. IS*285* and its insertional specificity

IS*285* is 1315 bp long, belongs to IS*256* family and is capable of choosing specific DNA sequences during its transposition. It results in regional and local specificity of IS*285* insertions in *Y. pestis* plasmid pLcr and presence of the majority of IS*285* copies in a "hot" region containing one third of the bacterial chromosome (Filippov *et al.,* 1990a; 1995; Filippov, 2001). This data a priori suggested a tendency of IS*285* to generate conservative chromosomal insertions that may be used for clustering. Comparison of five genome sequences showed that there are 19-25 copies of IS*285* in *Y. pestis* chromosome (Chain *et al.,* 2006) that is optimal for RFLP analysis. At the same time, IS*100* and IS*1541* are present in 30-75 and 47-67 copies, respectively. Such large numbers of copies impede the analysis of IS patterns. To overcome this problem, some investigators used PCR-based fingerprinting of few IS*100* insertions (Motin *et al.,* 2002), while others excluded high-molecular-mass bands from IS*100*- and IS*1541*-RFLP analyses (Torrea *et al.*, 2006).

3.2. Initial experiments on IS*285* typing

Our preliminary studies showed that IS*285* was present in the genomes of all 52 *Y. pestis* strains tested (Bobrov, Filippov, 1997). Chromosomal RFLP patterns of seven *Y. pestis* and nine *Y. pseudotuberculosis* strains suggested good clustering and discriminative abilities of IS*285*. In *Y. pestis* isolates, we could visualize 14-15 IS*285* bands, 10 of which were conservative, i.e. common for all strains belonging to biovars Antiqua, Medievalis and Orientalis. The rest of the fragments allowed to differentiate *Y. pestis* strains from each other. But when working with a homogenous group of *Y. pestis* strains of biovar Orientalis isolated in the United States, we observed insufficient discriminative power of IS*285*,

which produced only four clusters (Huang *et al.*, 2002). Better differentiation was found with IS*100*, and the optimal result was obtained by macrorestriction analysis using SpeI. However, recent publication (Torrea *et al.*, 2006) describing comparison of IS*100*, IS*285* and IS*1541* typing techniques tested for 61 *Y. pestis* strains of the three biovars showed that IS*285* provides easiest analysis, very good clustering and wholly satisfactory discrimination (38 IS types among 61 strains, better than that of multi-copy element IS*1541*).

3.3. We can trace *Y. pestis* global isolates with IS285

In this work, we used IS*285* as a probe for typing of a large unique collection of *Y. pestis* isolates from Europe (Russia, Armenia, Azerbaijan), Asia (Russia, Kazakhstan, Kirghizia, Turkmenistan, Tajikistan, Iran, Pakistan, China, Mongolia, India, Vietnam, Indonesia), Africa (Kenya, Algeria, Libya, Senegal, Congo, Madagascar), South (Brazil) and North (United States) Americas. A large part of the isolates were pestoides strains. Total cellular DNAs of 86 strains were cut with HindIII and hybridized with 571-bp EcoRV fragment of IS*285* labelled with DIG system (Boehringer Mannheim/Roche, Austria). The hybridization profiles were analyzed with BioNumerics software, version 4.0 (Applied Maths, Belgium).

Sixty-four IS*285*-RFLP types were elucidated among the 86 strains. IS*285* allowed us to establish distinct intraspecific taxons correlating with common geographical origin and species of animal carrier. Our data suggest a marked philogenetic isolation of pestoides strains from typical *Y. pestis* isolates. Five pestoides variants formed distinct clusters. Typical fully virulent *Y. pestis* strains displayed three large clusters corresponding to biovars Antiqua, Medievalis, and Orientalis. In some cases, it was possible to establish within these biovars smaller taxons, ecovars, associated with geographical areas (natural plague foci) and/or animal source. With the use of IS*285* typing, we were able to establish the origin of some pestoides strains that are used in molecular genetic experiments (A, B, C, D, E, and J.

4. CONCLUSIONS

IS*285* is a powerful instrument of *Y. pestis* genotyping and molecular epidemiology allowing the determination of evolutionary relationships and identification of differences between strains as well as to establish intraspecific taxons and to trace specific isolates. These features of IS*285* are especially important for identification of the origin of *Y. pestis* strains that may be applied as bioweapon or bioterrorism agents.

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